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Extraction of Quercetin from Citrus Sinensis using Ultrasound Assisted Hydrotropic Extraction

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Abstract:
Background: Quercetin, a bioactive flavonoid with significant antioxidant and anti-inflammatory properties, suffers from poor aqueous solubility, limiting its bioavailability.
Objective: This study aimed to enhance quercetin solubility and extraction efficiency from orange peels using ultrasound-assisted hydrotropic extraction (UAHE) with sodium benzoate.
Methods: The molar absorptivity of quercetin was determined via UV spectroscopy. Hydrotropic solubilization was performed at varying sodium benzoate concentrations (0–3 mol/L). Process parameters (hydrotrope concentration, extraction time, solid loading) were optimized using Response Surface Methodology (RSM).
Results: Maximum solubility (22.8×10^{-3} mol/L) was achieved at 2.6 mol/L hydrotrope concentration. Optimal UAHE conditions (3 mol/L, 30 min, 20% w/v) yielded 26.2 µg/g quercetin, with sodium benzoate outperforming sodium benzene sulphonate. RSM confirmed hydrotrope concentration as the most influential factor ($p < 0.05$).

Conclusion: UAHE with sodium benzoate is a green, efficient method for quercetin extraction, offering potential for pharmaceutical applications.

***Keywords*: Quercetin, Hydrotropic extraction, Ultrasound-assisted extraction, Sodium benzoate, orange peels, Response Surface Methodology**

1. INTRODUCTION

Quercetin, a flavonoid known for its antioxidant, anti-inflammatory, and therapeutic properties, is widely found in plant-based sources, including Citrus sinensis (sweet orange) peels. Due to its potential health benefits, quercetin has gained interest in the pharmaceutical, nutraceutical, and food industries. However, conventional extraction techniques, such as solvent-based extractions, often suffer from inefficiency, long processing times, and environmental concerns due to the use of toxic organic solvents. Ultrasound-assisted hydrotropic extraction (UAHE) offers a sustainable and efficient alternative for quercetin isolation. This study aims to optimize quercetin extraction using UAHE and evaluate its efficiency through UV-Vis spectroscopy. The application of hydrotropes enhances solubility, allowing for a greener and more effective extraction approach.

2. MATERIALS AND METHODS

2.1 Materials

- Chemicals: Sodium benzoate, sodium benzene sulphonate, quercetin, and acetone were procured from SRL and TCI India.
- Glassware: Conical flasks, beakers, pipettes, centrifuge tubes, and funnels.
- Equipment:
  - UV-Visible Spectrophotometer (Hitachi UV-200)
  - Magnetic Stirrer (Remi 1MLH)
  - Centrifuge (Remi R-24)
  - Ultrasonic Bath (Equitron)

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2.2 Molar Absorptivity Determination
Standard solutions of quercetin were prepared and analyzed at λmax = 278.5 nm using a UV-Vis spectrophotometer. Absorbance vs. concentration was plotted to determine molar absorptivity using Beer-Lambert's law.

2.3 Hydrotropic Solubilization
Hydrotrope solutions of various concentrations were prepared. A fixed amount of quercetin was added and stirred for 30 minutes. The solutions were filtered and analysed for solubility using UV-Vis spectroscopy.

2.4 Sample Preparation
Orange peels were dried for two days under atmospheric conditions and ground into powder using an electric blender. The powder was stored in an airtight container.

2.5 Ultrasound-Assisted Hydrotropic Extraction (UAHE)
A 50 mL hydrotrope solution (3 M) was mixed with 5 g of orange peel powder and placed in an ultrasonic bath for different time intervals. The extract was then stirred for 30 minutes, filtered, diluted with acidic water, and centrifuged. The precipitate was dissolved in acetone for UV analysis.

2.6 Optimization Using Response Surface Methodology (RSM)
Box–Behnken design was used to study the effects of hydrotrope concentration, extraction time, and solid loading on quercetin yield. The results were analyzed using regression models to determine optimal extraction conditions.

3. EXPERIMENTAL

3.1 Molar Absorptivity of Quercetin

Standard quercetin solutions of 10, 20, 30, 40, and 50 µg/mL were prepared. UV-Vis spectrophotometric analysis was conducted at λmax = 278.5 nm. Absorbance increased linearly with concentration, confirming Beer-Lambert's law. The calculated molar absorptivity was 13,001 $mol^{-1}cm^{-1}$, which aligns with literature values and supports method accuracy.

Table 3.1. Absorbance of Standard Quercetin Solutions

Concentration (µg/mL)	Absorbance
10	0.015
20	0.034
30	0.055
40	0.073
50	0.094

Using the linear equation from Beer-Lambert's law:
A = εcl
Where,
A = Absorbance
ε = Molar absorptivity (L mol^{-1} cm^{-1})
c = Concentration (mol/L)
l = Path length (1 cm)

From the slope of the calibration curve:
ε = 13,001 L mol^{-1} cm^{-1}

3.2 Hydrotropic Solubility Analysis
Sodium benzoate concentrations ranging from 0 to 3 mol/L were evaluated for their solubilization potential.
The following solubility values were recorded.

Table 3.2. Effect of Sodium Benzoate on Quercetin Solubility

Hydrotrope Concentration (mol/L)	Solubility ($\times 10^{-3}$ mol/L)
0	0.0002
0.2	0.0005
0.4	0.0037
0.6	0.0055
0.8	0.0076
1.0	0.0094
1.2	0.0110
1.4	0.0130
1.6	0.0150
1.8	0.0180
2.0	0.0200
2.2	0.0210
2.4	0.0223
2.6	0.0228
2.8	0.0228
3.0	0.0228

3.3 Ultrasound-Assisted Extraction Performance
Experiments were carried out with varying extraction times, solid loadings, and hydrotrope concentrations. Below is a sample dataset showcasing the variation in quercetin yield.

Table 3.3. Yield of Quercetin with Sodium Benzoate (UAHE)

Run	Hydrotrope (mol/L)	Time (min)	Solid Loading (% w/v)	Yield (µg/g)
1	3	30	20	26.6
2	3	20	30	19.9
3	1	10	20	11.9
4	3	20	10	25.6
...	...	...	...	...

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3.4 Optimization via RSM

The quadratic regression model derived for yield prediction using Box–Behnken Design is:

$$Y = -11.4 + 11.20X_1 - 0.074X_2 + 1.25X_3 - 1.32X_1^2 + 0.0005X_2^2 - 0.0255X_3^2 + 0.175X_1X_2 - 0.069X_1X_3 - 0.0118X_2X_3$$

Where:
Y = Quercetin yield (µg/g)
X_1 = Hydrotrope concentration (mol/L)
X_2 = Extraction time (min)
X_3 = Solid loading (% w/v)

The model was validated with $R^2 = 0.96$ for sodium benzoate and $R^2 = 0.97$ for sodium benzene sulphonate, confirming high reliability and fit with the experimental data.

4. RESULTS

4.1 Molar Absorptivity of Quercetin

The UV-Vis spectrophotometric analysis revealed a strong linear relationship between absorbance and quercetin concentration at a wavelength of 278.5 nm. The calibration curve resulted in a molar absorptivity value of 13,001 L mol^{-1} cm^{-1}, which is consistent with standard literature values, confirming the accuracy of the method.

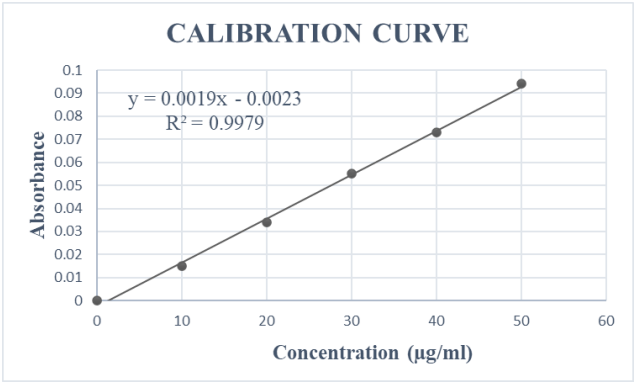

Figure 4.1. Calibration Curve of Quercetin

4.2 Effect of Hydrotrope Concentration on Solubility

The aqueous solubility of quercetin significantly increased with increasing sodium benzoate concentration. Below 0.2 mol/L (Minimum Hydrotropic Concentration, MHC), little solubility enhancement was observed. The solubility peaked at 2.6 mol/L (Maximum Hydrotropic Concentration, Cmax), beyond which no further increase was noted.

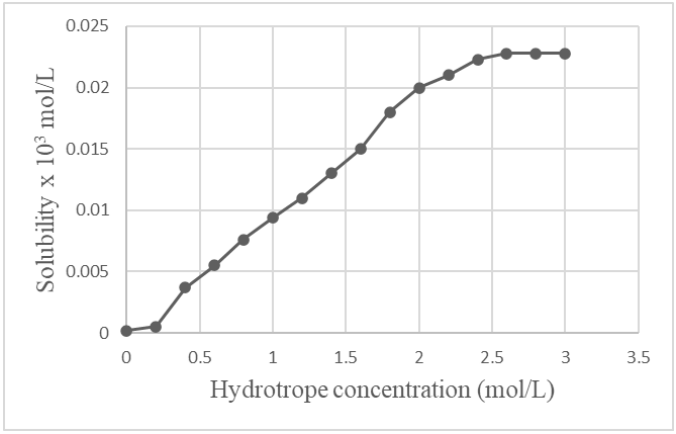

Figure 4.2. Solubility Profile of Quercetin with Sodium Benzoate Concentration

4.3 Ultrasound-Assisted Extraction Efficiency

The UAHE technique significantly enhanced quercetin yield compared to conventional methods. Optimal conditions for sodium benzoate (3 mol/L, 30 min, 20% w/v) yielded 26.6 µg/g of quercetin. Sodium benzene sulphonate under the same conditions yielded 19.2 µg/g. The improvement can be attributed to the combined action of ultrasound and hydrotropy, which enhances cell wall disruption and solute diffusion.

4.4 Response Surface Methodology Analysis

The Box–Behnken design was employed to evaluate the effects of hydrotrope concentration (X_1), extraction time (X_2), and solid loading (X_3) on yield. A second-order polynomial model was developed and showed excellent correlation ($R^2 = 0.96$ for sodium benzoate; $R^2 = 0.97$ for sodium benzene sulphonate). The model was validated through ANOVA.

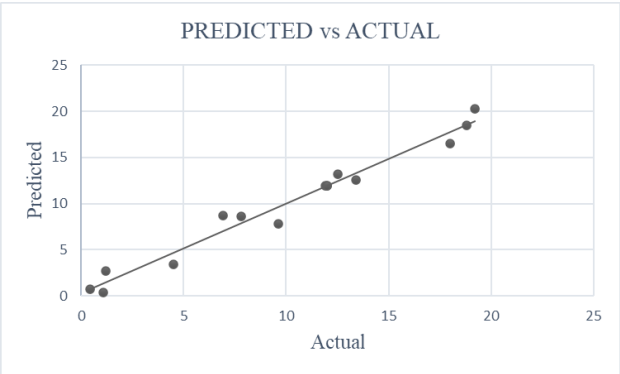

Figure 4.3. Predicted vs Actual Yield of Quercetin

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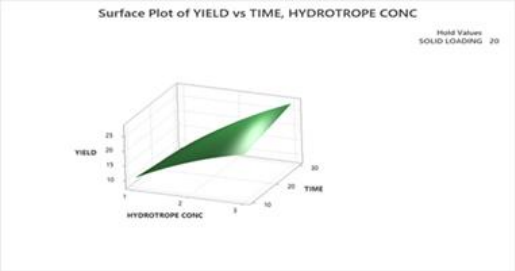

Figure 4.4. Response Surface Plot for Sodium Benzoate

4.5 Comparative Yield Performance

Table 4.1 compare the maximum experimental and predicted yields for both hydrotropes. Sodium benzoate demonstrated higher efficiency, confirming its suitability for UAHE.

Table 4.1. Comparison of Experimental and Predicted Yields

Variables	**Optimum Conditions (Sodium benzene sulfonate)**		**Optimum Conditions (Sodium benzoate)**	
Hydrotropic Concentration (mol)	3		3	
Extraction Time (min)	30		30	
Solid Loading (%w/v)	20		20	
Yield of quercetin (µg/g)	**Experimental**	**Predicted**	**Experimental**	**Predicted**
	19.2	20.2875	26.2	27.235

5. DISCUSSION

The findings of this study demonstrate the effectiveness of ultrasound-assisted hydrotropic extraction (UAHE) in enhancing the solubility and yield of quercetin from *Citrus sinensis* peels. The molar absorptivity value of 13,001 L mol^{-1} cm^{-1} obtained for quercetin confirms the reliability of UV-Vis spectrophotometry as a quantification method.

Hydrotropic solubilization using sodium benzoate was shown to significantly increase the solubility of quercetin. The determination of Minimum Hydrotropic Concentration (MHC) and Maximum Hydrotropic Concentration (Cmax) was crucial in defining the effective concentration range. The solubility sharply increased above the MHC (0.2 mol/L) and plateaued after reaching Cmax (2.6 mol/L), indicating that higher concentrations did not further improve solubilization.

Ultrasound-assisted extraction improved mass transfer and facilitated cellular disruption, thus enhancing extraction efficiency. The maximum yield of 26.6 µg/g obtained with sodium benzoate clearly outperformed sodium benzene sulphonate, which yielded 19.2 µg/g under the same conditions. The superior performance of sodium benzoate can be attributed to its better solubilizing capacity and compatibility with ultrasound.

Optimization via Response Surface Methodology (RSM) allowed for the systematic assessment of variable interactions. The model exhibited high R^2 values (0.96 and 0.97), indicating strong predictive power and model adequacy. Hydrotrope concentration had the most significant effect on extraction yield, followed by extraction time and solid loading.

CONCLUSION

The present study established ultrasound-assisted hydrotropic extraction (UAHE) as a novel and efficient approach for enhancing the solubility and extraction yield of quercetin from *Citrus sinensis* peels. The application of sodium benzoate as a hydrotropic agent significantly improved quercetin solubility, with optimal conditions identified at 3 mol/L hydrotrope concentration, 30 minutes extraction time, and 20% w/v solid loading. Comparative analysis indicated that sodium benzoate outperformed sodium benzene sulphonate, delivering a maximum experimental yield of 26.6 µg/g. The study also reaffirmed the importance of identifying Minimum Hydrotropic Concentration (MHC) and Maximum Hydrotropic Concentration (Cmax) for efficient solubilization. Process optimization using Response Surface Methodology (RSM) provided a statistically robust model with high predictive accuracy ($R^2 > 0.95$), supporting the reliability of the experimental design.

In conclusion, UAHE presents a sustainable, scalable, and environmentally friendly extraction technique with significant potential for the recovery of phytochemicals. Its application can be extended to other bioactive compounds, contributing to advancements in natural product extraction for pharmaceutical and nutraceutical development.

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